# Agilent MassHunter Workstation Software – Data Acquisition for 6410 Triple Quad LC/MS

## Familiarization Guide


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Use the exercise in this guide to learn to use the Agilent 6410 Triple Quad LC/MS. You can do these exercises with the demo data files, SulfaDrugs shipped with the system (in the **Data** folder of your Data Acquisition installation disk), or with data you acquire.

With this exercise, you learn how to determine the best acquisition settings for analyzing your compounds of interest. These instructions help you understand not only how to set up a worklist to optimize instrument parameters for best sensitivity in acquisition, but also how to use the Qualitative Analysis program to identify parameter values producing optimum signal response. You can also learn about the Qualitative Analysis program by using the *Qualitative Analysis Familiarization Guide*.

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In this exercise, you create an acquisition method for a mixture of four sulfa drugs, optimizing both the fragmentor and collision energy voltages to maximize sensitivity. One of the ways to optimize parameters is to create a worklist, or sequence, of data file acquisitions, each using a different method. This exercise uses this protocol for method development.

Another way to develop a method is to use the manual tune capability to optimize various parameters, including collision energy, to obtain the optimal signal response for each multiple reaction monitoring (MRM) transition. A third technique has you set up an acquisition method that directs the instrument to make multiple injections of the sample from an autosampler vial and acquire the data to a single data file. The method contains multiple time segments, one for each injection, with an incremental change made to a particular parameter (e.g., collision energy) in each segment.

This exercise uses the first protocol for method development.

**NOTE** See the *Concepts Guide* to learn more about how the triple quadrupole mass spectrometer works and why the fragmentor and collision energy voltages are important. For background information, see Chapter 3, "Agilent Triple Quad MS and Sensitivity", in the *Concepts Guide*. See the online Help for detailed information on how the program works.

Each task is presented in a table with three columns:

- Steps – Use these general instructions to proceed on your own to explore the program.
- Detailed Instructions – Use these if you need help or prefer to use a step-by-step learning process.
- Comments – Read these to learn tips and additional information about each step in the exercise.

# Before you begin

Before you begin, you need to check that your system is ready. If you plan to acquire data, you also need to set up the instrument.

## Prepare your system

1 Check that:

- The Data Acquisition program has been installed.
- The LC modules and the 6410 Triple Quad LC/MS have been configured.
- The performance has been verified.
- The system has been turned on.

If these actions have not yet been done, see the *Installation Guide* for your instrument.

2 Copy the data files to your PC.

Copy the folder named **SulfaDrugs** in the **Data** folder on your Qualitative Analysis installation disk to any location on your hard disk. This folder contains all the data files needed for this exercise.

NOTE Do not re-use the sulfa drug data files already on your system unless you know that you copied them from the originals on the disk and you are the only one using them.

Do not use these sample data files to look at sample information or print a report.

## Prepare to acquire data

If you do not intend to acquire data but want to learn how to use the Qualitative Analysis program for method development, you can skip this step, which tells you how to prepare the demo sample. You then do those tasks that show you how to use the Qualitative Analysis program with the sulfa drug data files shipped with the system.

**Parts List** The exercises in this guide use this equipment and materials:

- Agilent 1100 LC modules: well-plate sampler, binary pump, thermostatted column compartment, DAD
- Zorbax, SB-C18 2.1mm x 30mm, 3.5um, 100Å, p/n 873700-902.
- A 10-µL sulfa mix sample (prepared in this step)

1 Prepare the LC solvent.

   In 1-liter reservoirs of HPLC-grade water and acetonitrile (ACN), add 1 ml of 5M $NH_4HCO_2$ (Ammonium Formate) each to make 5mM $NH_4HCO_2$ in H2O and ACN and use for the A and B channels, respectively.

2 Prepare the sample.

   **a** Add 10 µL sulfa mix from one of the ampoules (500 µL) to 990 µL of solvent A in an Eppendorf vial so that the final concentration is 1 ng/µL.

   **b** Place a sample vial containing an injectable amount of the prepared sample in the autosampler.

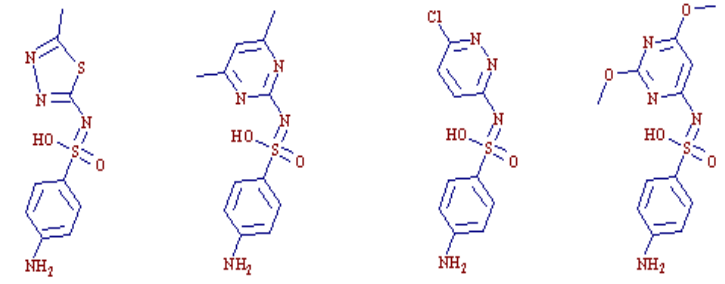
3 Set up the LC column.

   Use this Agilent column or equivalent: Zorbax, SB-C18 2.1mm x 30mm, 3.5um, 100Å, p/n 873700-902.

4 Set the column temperature.

   Agilent suggests a column temperature of 40°C, but this exercise can run at room temperature.

The Electrospray LC Demo Sample (P/N 59987-20033) contains five ampoules with 100 ng/µL each of sulfamethizole $(M+H)^+ = 271$, sulfamethazine $(M+H)^+ = 279$, sulfachloropyridazine $(M+H)^+ = 285$, and sulfadimethoxine $(M+H)^+ = 311$.




**NOTE** Determining optimal parameter values for acquiring sample compound data requires that the Agilent Triple Quad instrument already be tuned on the Tuning Mix calibrant ions. Before proceeding with this exercise, make sure you have used Checktune or Autotune to verify that calibrant ions each have the proper mass assignment, peak width, and signal intensity.

See the *Quick Start Guide* or online Help for instructions on tuning the instrument.

# Exercise – Develop an acquisition method for the 6410

For this exercise you analyze a mixture of four sulfonamide compounds.

## Task 1. Enter acquisition parameters and acquire data

In this exercise, you enter the conditions for the analysis of the sulfa drug mix.

| Steps | Detailed Instructions | Comments |
|---|---|---|
| **1** Enter LC parameters appropriate for sulfa drug mix.<br><br>See Table 1. | **a** Double-click the **Data Acquisition** icon.<br>**b** Make sure that Acquisition appears as the selection in the **Context** text box. If Tune is the selection, click **Acquisition** from the **Context** dropdown menu in the Combo bar.<br>**c** Enter the LC parameters listed in the Table 1. | • The Data Acquisition window appears. See Figure 1. |

**Table 1** LC parameters for sulfa drug mix

| Parameter | Value |
|---|---|
| **PUMP** | |
| • Flowrate | 800 µL/min |
| • Solvent A | 5 mM $NH_4HCO_2$ in $H_2O$ |
| • Solvent B | 5 mM $NH_4HCO_2$ in ACN |
| • Gradient (min - %B) | 0 min - 13%<br>1.80 min - 60%<br>2.50 min - 60% |
| • Stop Time | 2.50 min |
| • Post Time | 2.50 min |
| **INJECTOR** | |
| • Inj. Vol. | 1 µL |
| • Injection | Standard |

**Table 1** LC parameters for sulfa drug mix

| Parameter | Value |
|---|---|
| • Draw Position | 3.0 mm |
| **UV DETECTOR** | |
| • Ch A | 254 nm (4 nm BW on DAD) |
| • REF A (DAD only) | 400 nm (80 nm BW) |
| **COL THERM** | |
| • Temp | 40°C |

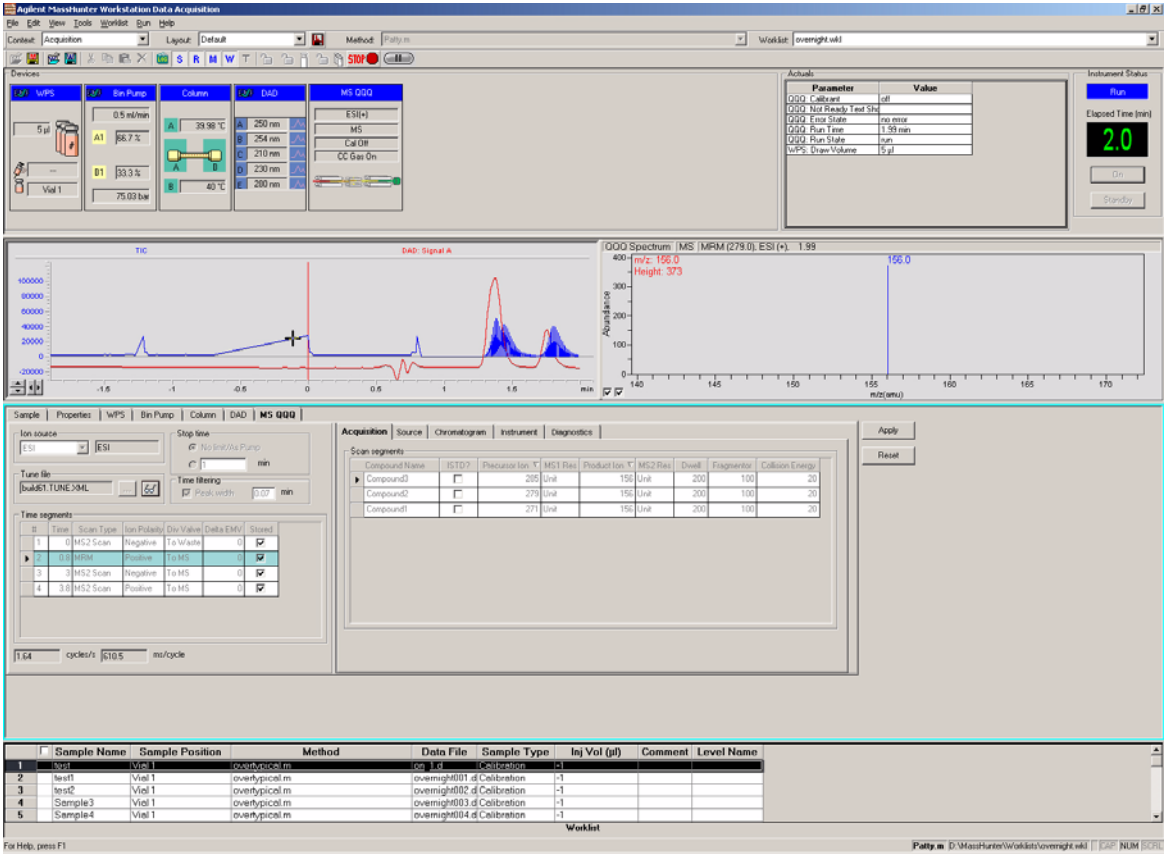



**Figure 1** Agilent MassHunter Workstation Software – Data Acquisition window

| Steps | Detailed Instructions | Comments |
|---|---|---|
| **2** Enter MS parameters appropriate for sulfa drug mix and save the method as ***iii*MS2Scantest.m**, where ***iii*** are your initials.<br><br>See Table 2. | **a** Click the **MS QQQ tab in the Method Editor pane.**<br>**b** Click the **Scan Type** cell, and click **MS2Scan** from the list.<br>**c** Enter the other MS parameters as listed in Table 2. These parameters are in either the Acquisition or the Source tabs.<br>**d** Save the method as ***iii*MS2Scantest.m**, where ***iii*** are your initials. | |

**Table 2** MS parameters for sulfa drug mix

| Parameter | Value |
|---|---|
| • Source | ESI (positive polarity) |
| • Gas Temp | 350 °C |
| • Scan Type | MS2Scan |
| • Nebulizer | 50 psi |
| • Dry Gas | 12 L/min |
| • Range | 100 to 400 |

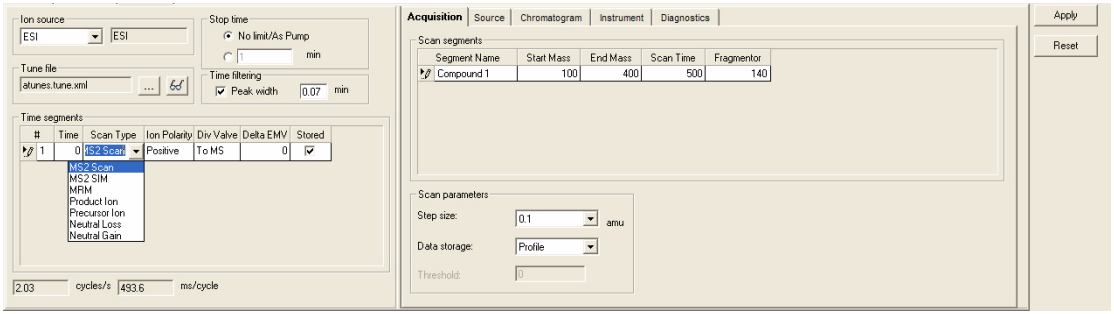
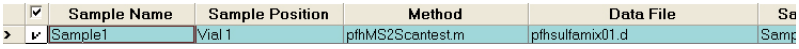


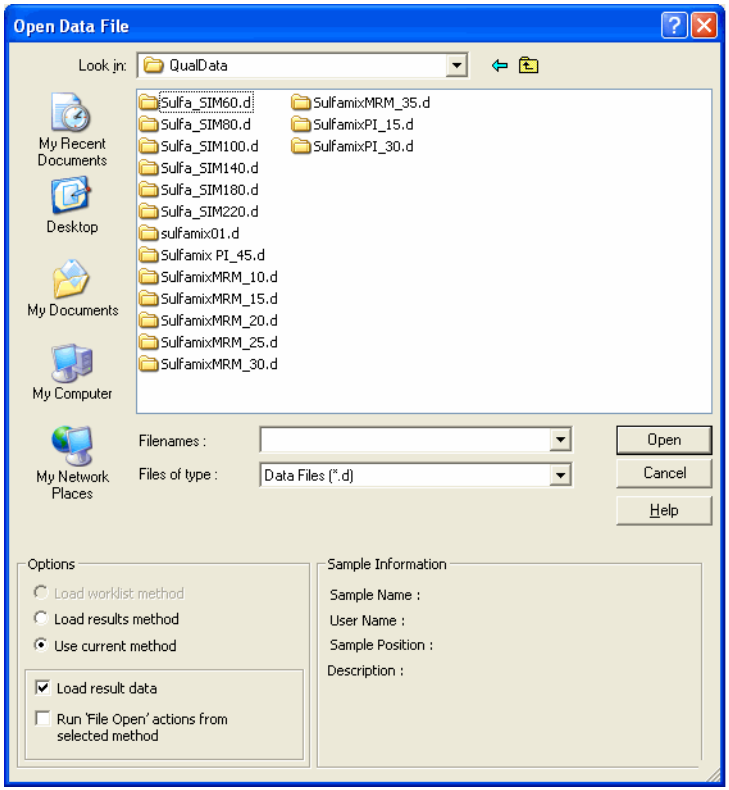

**Figure 2** Select **Scan Type** of MS2 Scan in the Acquisition tab

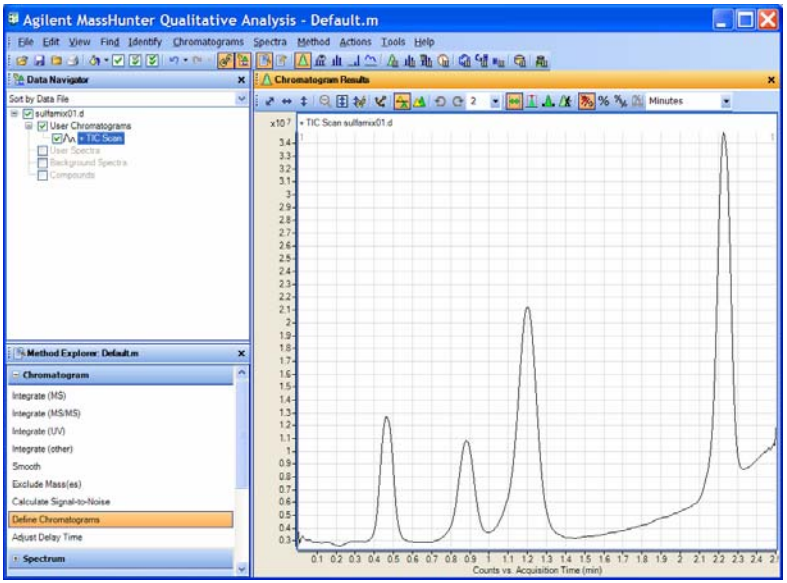
| Steps | Detailed Instructions | Comments |
|---|---|---|
| **3** Acquire data (optional).<br>• Set up a one-line worklist with the method you just created.<br>• Name the data file ***iii*sulfamix01.d**, where ***iii*** are your initials.<br>• Designate a directory path to hold your data files and method. | **a** If necessary, click the **Worklist** icon to display the Worklist pane.<br>**b** Click **Worklist > Worklist Run Parameters.** Verify that the parameters are set properly. Click **OK**.<br>**c** Click **Worklist > Add Multiple Samples**.<br>**d** Type `iiisulfamix01.d` and `iiiMS2Scantest.m` as the data file name and the method name, respectively.<br>**e** Click the Sample Position tab.<br>**f** Select **None** as the autosampler.<br>**g** Type 1 as the **Number of samples**.<br>**h** Click **OK.**<br>**i** In the Worklist pane, mark the check box to the left of the sample as shown below.<br>  <br>**j** Click the **Start Worklist Run** icon in the main toolbar or click the **Run > Worklist** menu item. | • You have just acquired a full scan MS data file to see what ions are being formed from the sample.<br>• This step is optional because you can perform the next step with an example data file that comes with the program. If you prefer, you can create your own data file as described in this step. |

## Task 2. Determine precursor ion masses

In this exercise, you determine the precursor ions for each of the sulfa drugs in the acquired data file.

| Steps | Detailed Instructions | Comments |
|---|---|---|
| **1** Open the acquired data file.<br>• In the Qualitative Analysis program, open either the example file, **sulfamix01.d,** or the data file you created in "Task 1. Enter acquisition parameters and acquire data" on page 5. | **a** Double-click the **Qualitative Analysis** icon. <br>The program displays the Open Data File dialog box. | • When you open the sulfa drug directory after installation, the **Load result data** (lower left corner) check box is grayed out.<br>• If you see the check box marked, this means that the data file(s) already contains results. ***Clear this check box before opening the file***. |

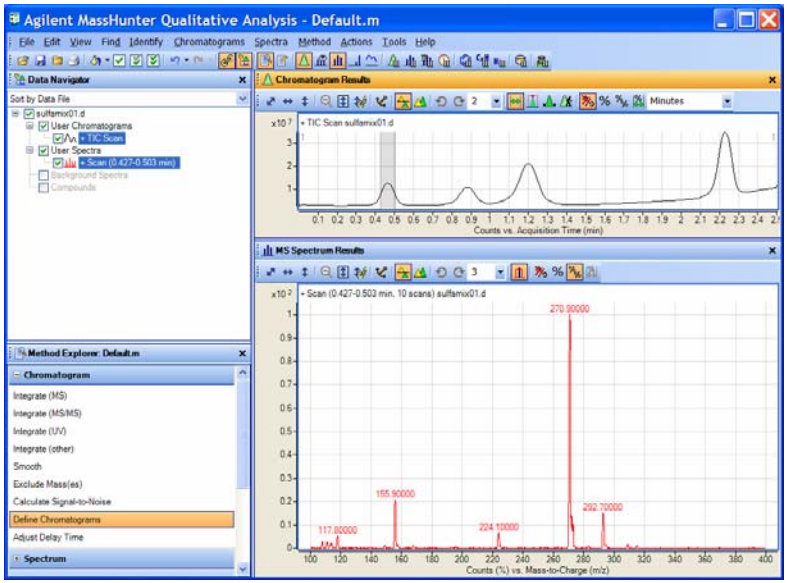


| Steps | Detailed Instructions | Comments |
|---|---|---|
| | **b** Do one of the following:<br>• Select the example data file **sulfamix01.d**, and click **Open**.<br>• Select the data file you created in "Task 1. Enter acquisition parameters and acquire data" on page 5, and click **Open**.<br>By default, the system displays the Total Ion Chromatogram (TIC). | • The figure below shows the default layout. This is what you want to see.<br>• The Qualitative Analysis program displays a newly opened data file with the same layout and display settings used for the previous data file. Therefore, you MUST make sure to return to the default settings for this exercise. |
| Before you begin, make sure that all previous settings are returned to their default values:<br>• Restore default layouts<br>  • Click **View** > **Window Layouts > Restore Default Layout**.<br>• Make sure the method is **default.m**. (see title bar)<br>  • Click **Method > Open**.<br>  • Select **default.m**, and click **Open**.<br>• Return display options to default settings.<br>  • Click **Tools > Plot Display Options...**<br>  • Click **Default**, and then **OK**. |  | |

| Steps | Detailed Instructions | Comments |
|---|---|---|
| **2** Determine precursor ion masses for all four peaks.<br>• You have determined them correctly if you find the values are similar to those shown in this table: | **a** **In the Chromatogram Results window, make sure that the Range Select icon in the toolbar is On.**<br>**b** Click the left mouse button and drag the cursor across the first peak to produce a shaded region, as in the figure below.<br>**c** Right-click the shaded area, and click **Extract MS Spectrum** from the shortcut menu. | • The system displays an averaged spectrum across the peak in the MS Spectrum Results window.<br>• The precursor mass of the first compound, sulfamethizole, is determined to be m/z 270.9.<br>• To obtain a single scan, double-click the apex of the peak. |

| Compound | RT | *m/z* |
|---|---|---|
| Sulfamethizole | 0.47 | 270.9 |
| Sulfachloropyridazine | 0.88 | 284.9 |
| Sulfamethazine | 1.20 | 279.0 |
| Sulfadimethoxine | 2.23 | 311.0 |

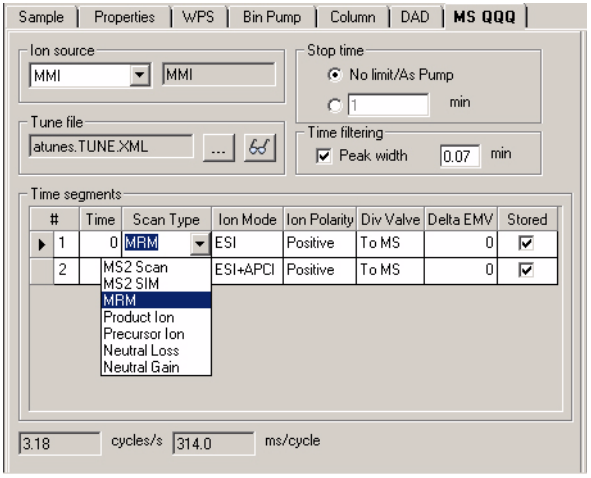
• Close the data file after finding the precursor ion masses.



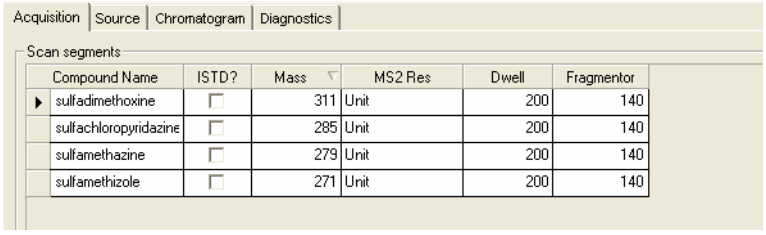

| | |
|---|---|
| **d** Repeat step a through step c for the other compounds.<br>The precursor ion masses should match those in the table in step 2.<br>**e** Click **File > Close Data File**.<br>**f** When asked if you want to save the results, click **No**. | • Some compounds form sodium (Na) and/or potassium (K) adducts as well, corresponding to M + 23 and M + 39 masses respectively. Seeing these masses along with the M + H can make for an easy confirmation of which ion is the pseudo-molecular ion (M + H)+. |

## Task 3. Find optimum fragmentor voltage for maximum response

Task 3 shows you how to carry out the optimization for fragmentor voltage by creating selected ion-monitoring experiments for each compound within a method and setting up multiple methods with varying fragmentor voltages.

| Steps | Detailed Instructions | Comments |
|---|---|---|
| **1** Set up six methods for six different fragmentor voltages.<br>• Change to a SIM experiment.<br>• Use 60, 80, 100 and 140, 180 and 220 volts as the fragmentor voltages for the six methods.<br>• Save the methods as ***iii*MS2SIM*xxx*.m**, where ***iii*** are your initials and ***xxx*** is the voltage. | **a** In the **Scan Type** dropdown list, click **MS2 SIM**.<br>  | |

| Steps | Detailed Instructions | Comments |
|---|---|---|
| | **b** In the **Acquisition** tab, enter the **Compound Name** and **Mass** (precursor ion mass) for sulfadimethoxine.<br>**c** Right-click anywhere in the Scan segments section, and click **Add Row**.<br>**d** Enter **Compound Name** and **Mass** for sulfachloropyridazine.<br>**e** Repeat steps c and d for sulfamethazine and sulfamethizole.<br>**f** **Save the method as *iii*MS2SIM140.m**, where ***iii*** are your initials**.**<br>**g** Change the fragmentor voltage to 60, and save the method as ***iii*MS2SIM060**, where ***iii*** are your initials.<br>**h** Repeat step g for voltages 80, 100, 180 and 220, saving the methods as ***iii*MS2SIM080, *iii*MS2SIM100, *iii*MS2SIM180 and *iii*MS2SIM220,** where ***iii*** are your initials. | • With the MS2SIM Scan Type set, a different set of columns appears in the Acquisition window.<br>• The Instrument Control and Data Acquisition program creates a SIM experiment for each compound mass, starting with a default fragmentor voltage of 140. See the example below. |

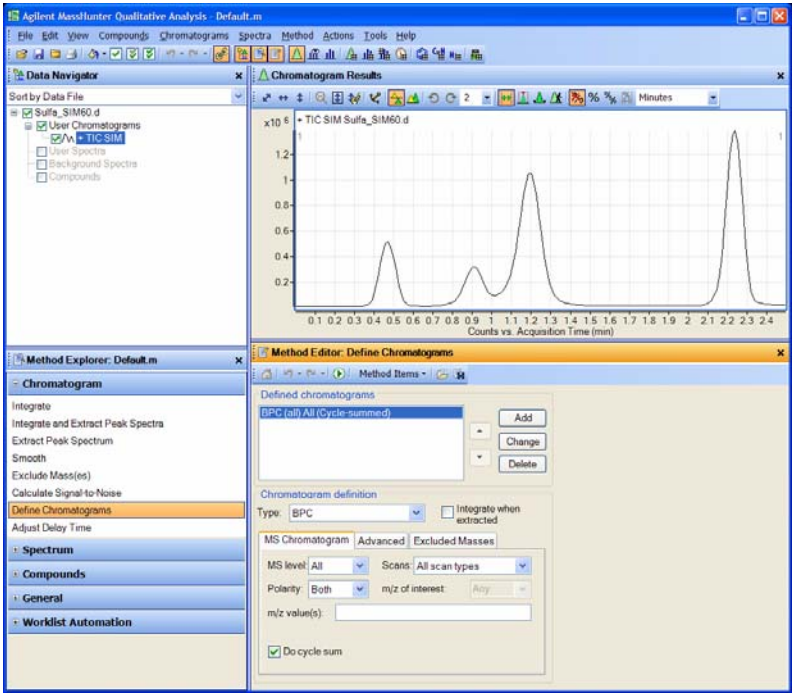


| Steps | Detailed Instructions | Comments |
|---|---|---|
| **2** Set up and run the worklist (optional).<br>• Set up six samples with Sample Name SulfaDrugMix to inject 1ul from vials 1-6 or the ones you choose.<br>• Specify the data files as ***iii*SulfaSIM*xxx*.d**, where ***iii*** are your initials and ***xxx*** is the voltage. | **a** Click the **Worklist** icon if necessary to make sure the worklist is visible.<br>**b** Click **File > New > Worklist** to start a new worklist. You do not need to save the last worklist.<br>**c** To set up the run, right-click the upper left corner of the worklist, and click **Worklist Run Parameters**.<br>**d** Type the paths for the method and data files.<br>**e** Type the information for the 60 voltage run.<br>**f** Click **Worklist > Add Sample**. Another sample is added to the Worklist. Add five samples to the worklist for voltages 80-220.<br>**g** Mark the checkbox to the left of the Sample Name for each of the six samples. | • This step is optional because you can use data files shipped with the system to perform many of the tasks in this exercise. |

| | ✓ | Sample Name | Sample Position | Acq Method | Data File | Sample Type |
|---|---|---|---|---|---|---|
| 1 | ✓ | SulfaDrugMix | Vial 1 | MS2SIM060.m | d:\Sulfa_SIM060.d | Sample |
| 2 | ✓ | SulfaDrugMix | Vial 1 | MS2SIM080.m | d:\Sulfa_SIM080.d | Sample |
| 3 | ✓ | SulfaDrugMix | Vial 1 | MS2SIM100.m | d:\Sulfa_SIM100.d | Sample |
| 4 | ✓ | SulfaDrugMix | Vial 1 | MS2SIM140.m | d:\Sulfa_SIM140.d | Sample |
| 5 | ✓ | SulfaDrugMix | Vial 1 | MS2SIM180.m | d:\Sulfa_SIM180.d | Sample |
| 6 | ✓ | SulfaDrugMix | Vial 1 | MS2SIM220.m | d:\Sulfa_SIM220.d | Sample |

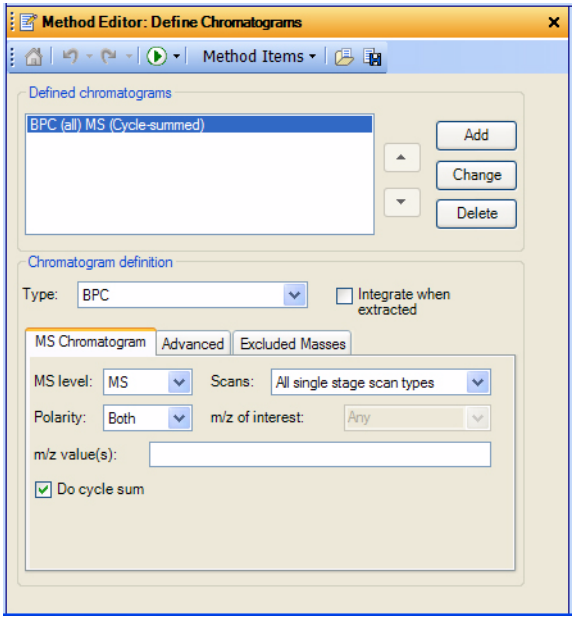
| | | |
|---|---|---|
| | **h** Click **Run > Worklist**. | • Note that the program only runs those samples that are enabled with a checkmark. |

| Steps | Detailed Instructions | Comments |
|---|---|---|
| **3** Set up a qualitative method to view the EIC data automatically.<br>• Open the data file **Sulfa_SIM60.d** or your own ***iii*Sulfa_SIM60.d**, where ***iii*** are your initials.<br>• In the Method Editor, add in the EICs corresponding to the precursor ion masses of 271, 279, 285, and 311.<br>• Save the method as *iii*Exercise1, where "*iii*" are your initials. | **a** Click **File > Open Data File**. The system displays the Open Data File dialog box<br>**b** Select either **Sulfa_SIM60.d** or ***iii*Sulfa_SIM60.d**, and click **Open**.<br>. | • The Qualitative Analysis program should be open. If not, see step 1 of Task 1 in this exercise. |




**c** Click **Method > View Method Editor**. The system displays the Method Editor window. (See figure on next page.)

| Steps | Detailed Instructions | Comments |
|---|---|---|
| | **d** If necessary, click **Define Chromatograms** from the Method Items pull-down menu. | • The default Method Editor list selection after installation is **Integrate**. |




**e** To delete the BPC chromatogram, click **Delete**.

**f** For the Chromatogram Definition Type, click **EIC**.

**g** In the MS Chromatogram tab, make sure **MS Level** is set to **All** and **Scans** is set to **All Scan Types**.

**h** Clear the **Do cycle sum** check box.

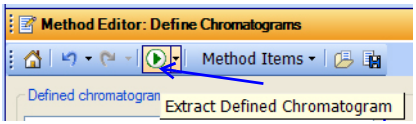
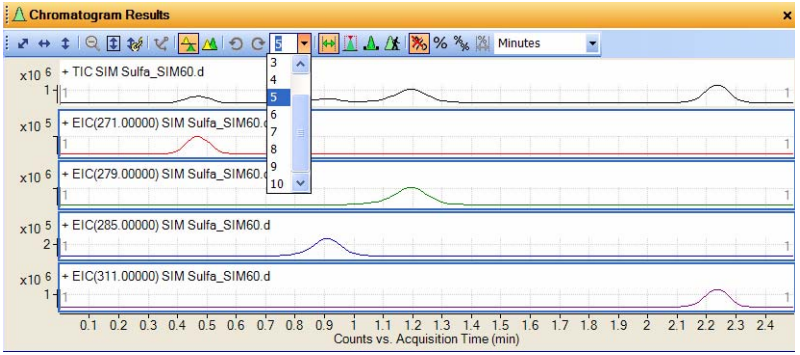
**i** Type 271 as the **m/z value**.

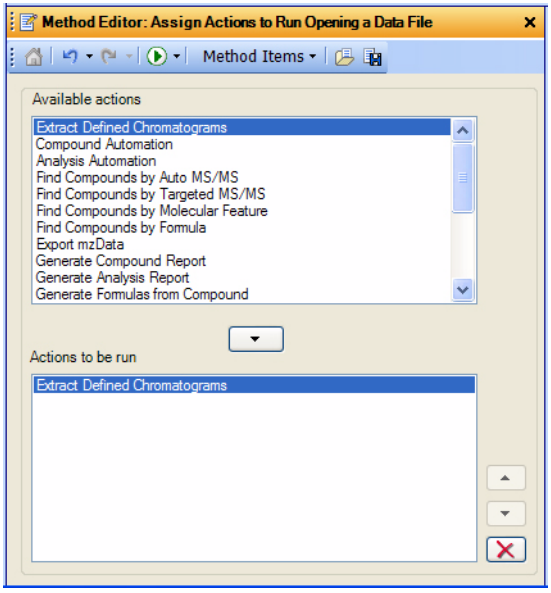
**j** Click **Add**.

**k** Repeat steps i and j for the other precursor ions, 279, 285 and 311.

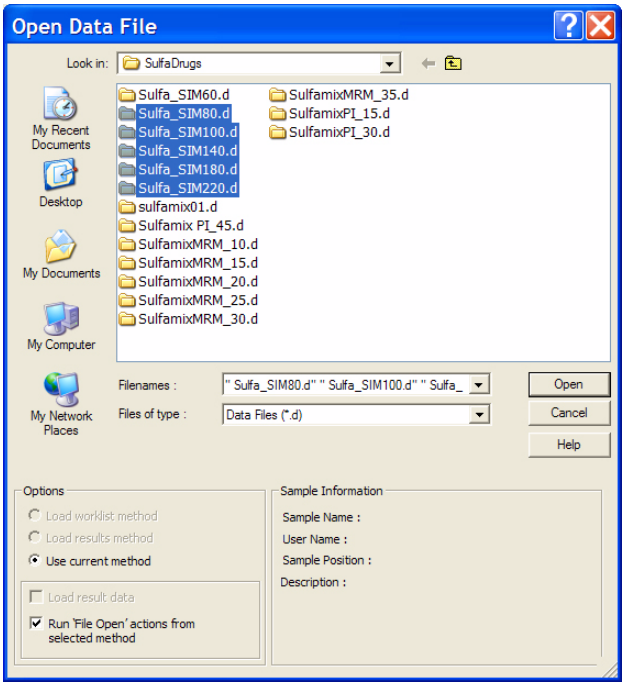
**l** Click **Method > Save As**.
The system opens the Save As dialog box.

**m** Save the method as ***iii*Exercise 1.m.**

| Steps | Detailed Instructions | Comments |
|---|---|---|
| **4** Extract the chromatogram for the data file and view the results.<br>• Make sure you can see all five chromatograms, the TIC and four EICs. | **a** To apply the method settings to the data file, click the **Extract Defined Chromatogram** icon on the toolbar.<br>  <br><br>**b** To see the TIC and four EICs, click the arrow next to the Maximum Number of List Panes icon in the Chromatogram Results toolbar, as shown in the example below.<br>**c** Select **5** to view five chromatograms simultaneously.<br>The system displays chromatogram results as shown below.<br>  <br> | |

| Steps | Detailed Instructions | Comments |
|---|---|---|
| **5** Extract the remaining ion chromatograms automatically.<br>• Extract Defined Chromatograms should be the default action for Assign File Open Actions.<br>• Open the remaining data files, Sulfa_SIM80.d through Sulfa_SIM220.d.<br>• Close the Method Explorer. | **a** Select **File Open Actions** from the General section in the Method Explorer.<br>**b** Make sure that **Actions to be run** is set to **Extract Defined Chromatograms.**<br>  <br>**c** Click **File > Open Data File.** The system displays the Open Data File dialog box.<br>**d** Select the data files to be opened, Sulfa_SIM80.d through Sulfa_SIM220.d.<br>**e** Mark the **Run 'File Open' actions from selected method** checkbox. (lower left corner) | • The Qualitative Analysis Method Editor lets you define actions to be performed automatically upon opening a data file(s). |

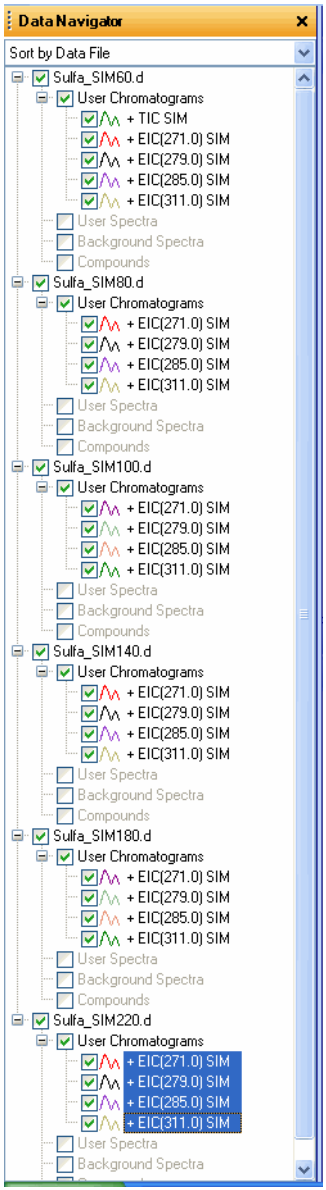
| Steps | Detailed Instructions | Comments |
|---|---|---|

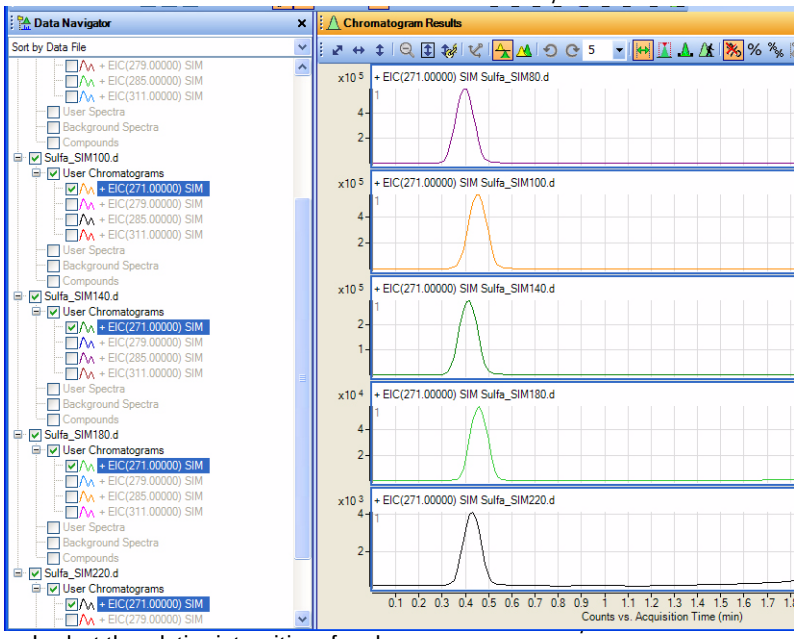



**f** Click **Open.**
The Qualitative Analysis program displays all the EICs for all the data files selected.

**g** To close the Method Explorer and Method Editor, click the **X** in the upper right corner of each window.

| Steps | Detailed Instructions | Comments |
|---|---|---|

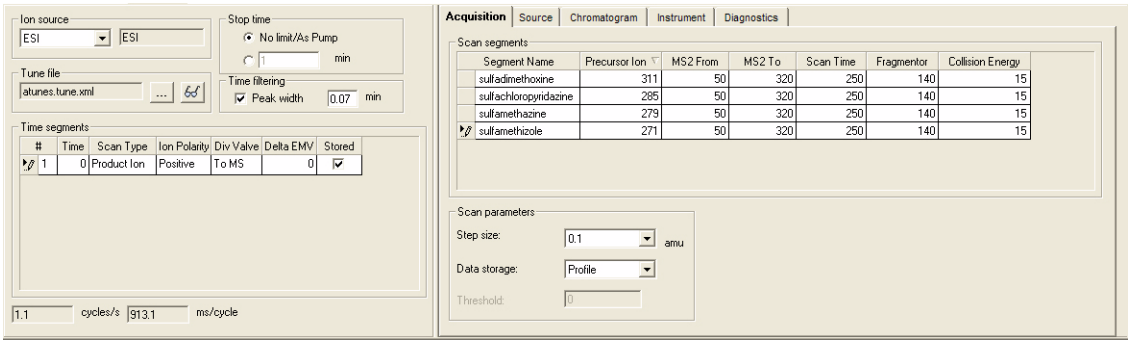


| Steps | Detailed Instructions | Comments |
|---|---|---|
| **6** Select the fragmentor voltage that produces the maximum response for each of the precursor ions.<br>• Close the data files after you determine the optimum voltage. | **a** In the Data Navigator window, highlight the EICs for 271.0 m/z.<br>**b** Click the **Show only the highlighted items** icon, .<br>Only the 271 m/z check boxes are now marked.<br>  <br>**c** Look at the relative intensities of each peak to determine which fragmentor voltage setting will be best to use for the 271 precursor.<br>**d** Repeat steps a-c for the other three base peaks or precursor ions.<br>**e** Click **File > Close Data File**.<br>**f** Click **Close** when the Close Data File dialog box appears. | • You press the **Ctrl** key to be able to select multiple files from the list You press the **Shift** key to be able to select a group of files.<br>• A fragmentor voltage of 100 should be sufficient for each precursor ion.<br>• You can now determine the product ions that are available for the multiple-reaction monitoring experiments to maximize sensitivity for the analysis.<br>chromatograms, by clicking the **Overlaid mode** icon in the Chromatogram Results toolbar. |

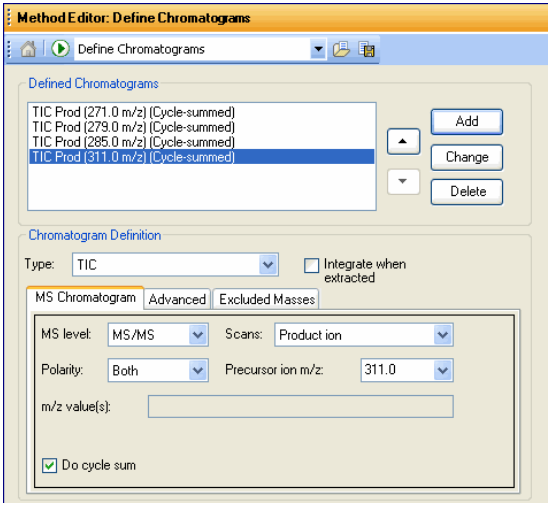
## Task 4. Determine product ion masses

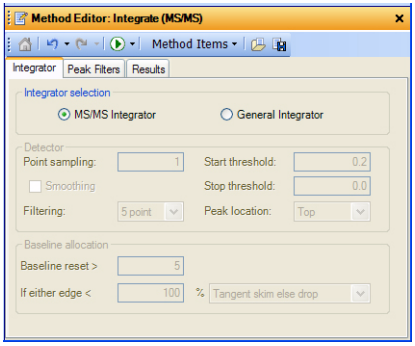
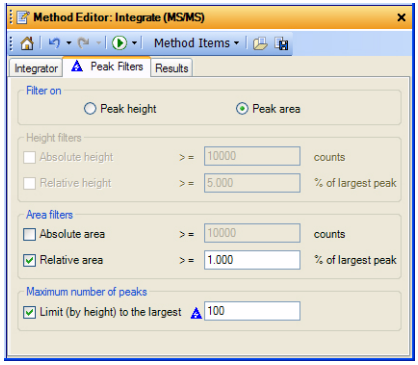
In this part of the method development, we will use three collision energies to determine the best fragment ions to use for the eventual Multiple Reaction Monitoring (MRMs).

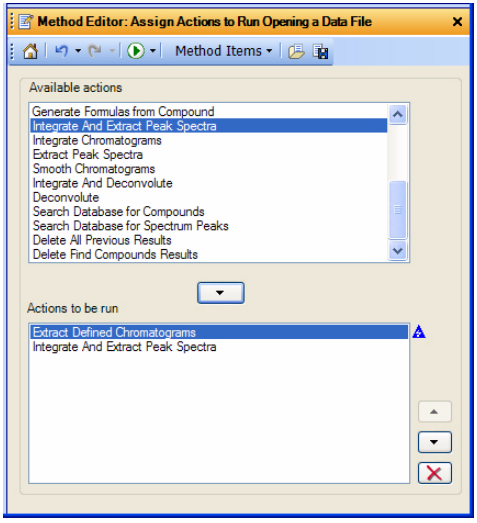
| Steps | Detailed Instructions | Comments |
|---|---|---|
| **1** Set up three product ion acquisition methods and acquire data.<br>• Use the MS parameters in the example below, but change the Fragmentor voltage to the optimum voltage you determined in the previous task.<br>• Save methods as ***iii*Sulfamix PI_*xx*.m**, where ***iii*** are your initials and ***xx*** is the collision energy. | **a** Click the **MS QQQ** tab in the Method Editor pane.<br>**b** Select **Product Ion** in the **Scan Type** field to scan each precursor ion for all its product ions.<br>**c** Enter all MS parameters as listed in the example below, making sure the **Collision Energy** is set to `15` and the Fragmentor voltage is set to the optimum voltage determined in Task 3.<br>**d** Save the method as ***iii*Sulfamix PI_15.m**.<br>**e** Repeat step c and step d for collision energies of 30 and 45. | |




| | | |
|---|---|---|
| **2** Set up and run the worklist (optional).<br>• Specify the data files as ***iii*Sulfamix PI_*xx*.d**, where ***iii*** are your initials and ***xx*** is the collision energy. | **a** Scroll down if necessary to make sure the worklist is visible.<br>**b** Add three samples to the worklist for collision energies 15, 30 and 45.<br>**c** Mark the checkbox to the left of the Sample Name for each sample you are adding.<br>**d** Click **Run > Worklist**. | • This step is optional because you can determine the product ion masses from the data files shipped with the system.<br>• Use the instructions in Step 2 of Task 3 to set up the worklist. |

| Steps | Detailed Instructions | Comments |
|---|---|---|
| **3** Set up a qualitative method to integrate and extract product ion spectra.<br>• Use the data files **SulfamixPI_*xx*.d,** where *xx* is the collision energy, or your own data files, ***iii*SulfamixPI_*xx*.d**.<br>• Open Method Explorer and Method Editor.<br>• Use TICs set up for MS/MS, product ion and each of the precursor ions 271, 279, 285, 311.<br>• Make sure the MS/MS integrator has been selected and the maximum number of peaks has been limited to the largest 100 peaks.<br>• Add the ability to integrate and extract peak spectra to the file actions run upon data opening.<br>• Save the changes to the current method. | **a** Click the **Open Data File** icon in the toolbar.<br>**b** Select **SulfamixPI_15.d**.<br>**c** Make sure that the **Run File Open Actions from Specified Method** check box is clear, and click **Open**.<br>**d** Make sure the Method Explorer and the Method Editor windows are displayed; otherwise, click the **Method Explorer** and then **Method Editor** icons.<br>**e** Under Chromatograms in Method Explorer, click **Define Chromatograms.**<br>**f** Delete any existing chromatograms in the Defined Chromatograms box.<br>**g** Select **TIC** from the **Chromatogram Definition** list.<br>**h** For **MS Level**, click **MS/MS**.<br>**i** For **Scans**, click **Product ion**.<br>**j** For **Precursor ion m/z**, type `271`.<br>**k** Click **Add**.<br>**l** Repeat steps j and k for each ion.<br>  | • The Qualitative Analysis program should already be open and contain *iii*exercise 1.m as the method. |

| Steps | Detailed Instructions | Comments |
|---|---|---|
| | **m** From the Method Explorer in the Chromatogram section, click **Integrate (MS/MS)**.<br>**n** Select the **MS/MS Integrator**, if necessary.<br>  <br>**o** Click the **Peak Filters** tab. Make sure that the **Limit to the largest** check box is marked and set to the value `100` (peaks) as shown below.<br>  <br>**p** Click **General** in Method Explorer, and then click **File Open Actions**.<br>**q** Select **Integrate and extract peak spectra** from the Available actions list and click to add this to **Actions to be run**. | • These data files contain MS/MS data, so you need to modify the parameters in the Integrate (MS/MS) section. If the data file contained only MS data, you would need to modify the parameters in the Integrate (MS) section. |

| Steps | Detailed Instructions | Comments |
|---|---|---|
| |  | |
| | **r** To apply the changes to the current method, *iii*exercise1.m, click the **Save Method** icon. | |
| **4** Run the qualitative method on the current data file. | • In the Method Editor toolbar, click the **Run** button, . When the Assign Actions to Run Opening A Data File section is displayed, the **Actions to be run** list is done. | • The program first extracts the product ion chromatograms for each precursor ion in the data file.<br>• Next, it finds the largest peak in the total ion chromatograms, and integrates and extracts peak spectra from each integrated peak.<br>• See Figure 3 on page 27. |

| Steps | Detailed Instructions | Comments |
|---|---|---|

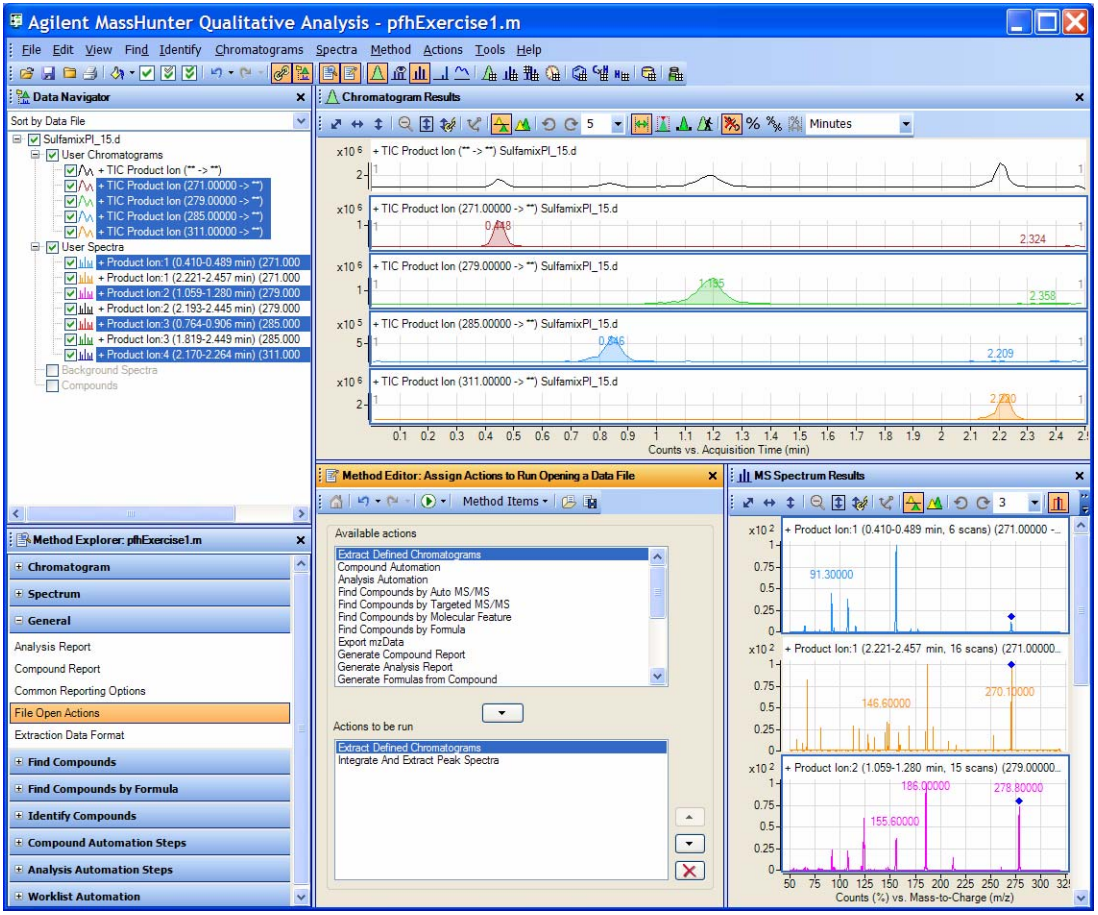
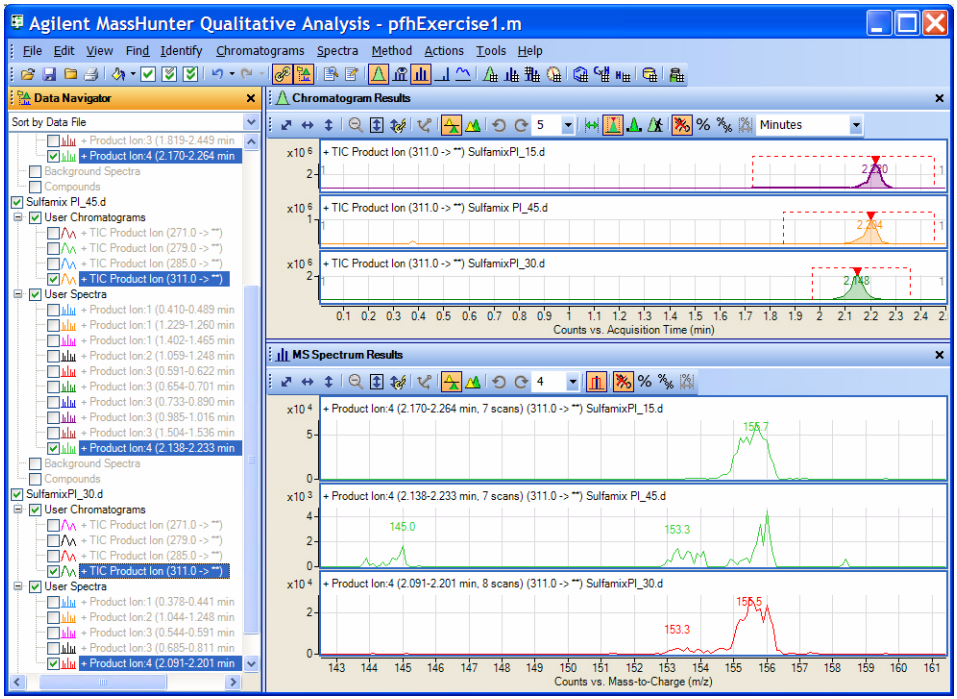

Agilent MassHunter Qualitative Analysis - pfhExercise1.m
File Edit View Find Identify Chromatograms Spectra Method Actions Tools Help
Data Navigator
Sort by Data File
SulfamixPI_15.d
User Chromatograms
+ TIC Product Ion (** -> **)
+ TIC Product Ion (271.00000 -> **)
+ TIC Product Ion (279.00000 -> **)
+ TIC Product Ion (285.00000 -> **)
+ TIC Product Ion (311.00000 -> **)
User Spectra
+ Product Ion:1 (0.410-0.489 min) (271.000
+ Product Ion:1 (2.221-2.457 min) (271.000
+ Product Ion:2 (1.059-1.280 min) (279.000
+ Product Ion:2 (2.193-2.445 min) (279.000
+ Product Ion:3 (0.764-0.906 min) (285.000
+ Product Ion:3 (1.819-2.449 min) (285.000
+ Product Ion:4 (2.170-2.264 min) (311.000
Background Spectra
Compounds
Chromatogram Results
Minutes
+ TIC Product Ion (** -> **) SulfamixPI_15.d
+ TIC Product Ion (271.00000 -> **) SulfamixPI_15.d
+ TIC Product Ion (279.00000 -> **) SulfamixPI_15.d
+ TIC Product Ion (285.00000 -> **) SulfamixPI_15.d
+ TIC Product Ion (311.00000 -> **) SulfamixPI_15.d
Counts vs. Acquisition Time (min)
Method Explorer: pfhExercise1.m
Chromatogram
Spectrum
General
Analysis Report
Compound Report
Common Reporting Options
File Open Actions
Extraction Data Format
Find Compounds
Find Compounds by Formula
Identify Compounds
Compound Automation Steps
Analysis Automation Steps
Worklist Automation
Method Editor: Assign Actions to Run Opening a Data File
Method Items
Available actions
Extract Defined Chromatograms
Compound Automation
Analysis Automation
Find Compounds by Auto MS/MS
Find Compounds by Targeted MS/MS
Find Compounds by Molecular Feature
Find Compounds by Formula
Export mzData
Generate Compound Report
Generate Analysis Report
Generate Formulas from Compound
Actions to be run
Extract Defined Chromatograms
Integrate And Extract Peak Spectra
MS Spectrum Results
+ Product Ion:1 (0.410-0.489 min, 6 scans) (271.00000 -...
91.30000
+ Product Ion:1 (2.221-2.457 min, 16 scans) (271.00000...
146.60000
270.10000
+ Product Ion:2 (1.059-1.280 min, 15 scans) (279.00000...
186.00000
278.80000
155.60000
Counts (%) vs. Mass-to-Charge (m/z)

**Figure 3** Results for integration and extraction of peak spectra.

| Steps | Detailed Instructions | Comments |
|---|---|---|
| **5** Run the 'File Open' actions on the remaining product ion data files.<br>• Use either the example files, **Sulfamix PI_*xx*.d**, or the data files you acquired in step 2. | **a** Click **File > Open Data File.** The system displays the Open Data File dialog box.<br>**b** Hold the **Ctrl** key and do one of these:<br>• Select the two data files **Sulfamix PI_30.d,** and **Sulfamix PI_45.d**.<br>• Select the data files you acquired in step 2.<br>**c** Mark the **Run 'File Open' actions from selected method** check box in the Open Data File dialog box, and click **Open**. | • After the data files open, the Qual method first extracts the product ion chromatograms for each precursor ion.<br>• Next, it finds the largest peak in the total ion chromatograms, and integrates and extracts peak spectra from each integrated peak.<br>• See the figure in step 6. |

| Steps | Detailed Instructions | Comments |
|---|---|---|
| **6** Identify product ions.<br>• View each set of TICs and spectra individually (e.g., 271 m/z first).<br>• Close the data files. | **a** In the Data Navigator, select the TICs and spectra for the 271 m/z precursor ion.<br>**b** Click the **Show only the highlighted items** icon, .<br>**c** Examine the spectra to see which fragment ions are produced at which collision energies.<br>**d** Repeat steps a-c until all the product ions are identified. | • The m/z 155.7 product ion is the most abundant of any product ion and the highest signal is recorded at 15 V. This means that a good choice for the MRM for sulfamethizole would be 271.0 > 155.7 when the collision energy is around 15 V.<br>• The peak may not be labeled if the peak is too wide. |

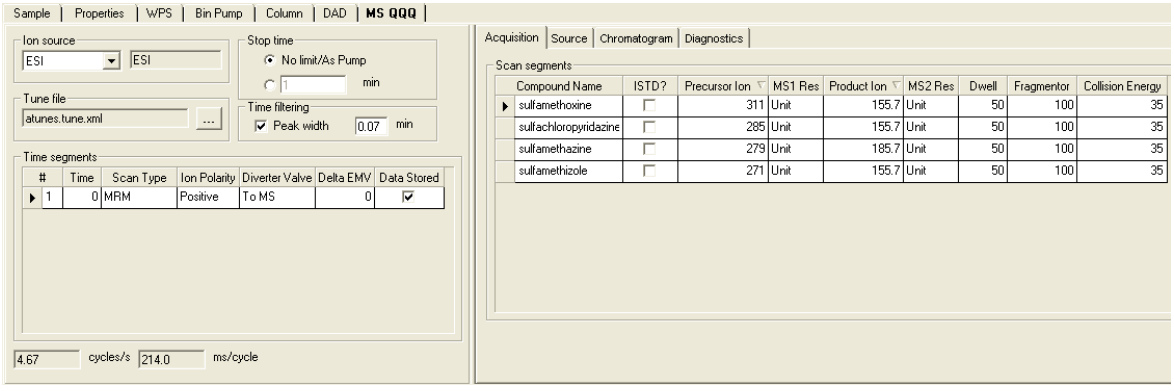

Agilent MassHunter Qualitative Analysis - pfhExercise1.m
File Edit View Find Identify Chromatograms Spectra Method Actions Tools Help
Data Navigator
Chromatogram Results
MS Spectrum Results
+ TIC Product Ion (311.0 -> **) SulfamixPI_15.d
+ TIC Product Ion (311.0 -> **) Sulfamix PI_45.d
+ TIC Product Ion (311.0 -> **) SulfamixPI_30.d
Counts vs. Acquisition Time (min)
+ Product Ion:4 (2.170-2.264 min, 7 scans) (311.0 -> **) SulfamixPI_15.d
+ Product Ion:4 (2.138-2.233 min, 7 scans) (311.0 -> **) Sulfamix PI_45.d
+ Product Ion:4 (2.091-2.201 min, 8 scans) (311.0 -> **) SulfamixPI_30.d
155.7
145.0
153.3
Counts vs. Mass-to-Charge (m/z)

| | | |
|---|---|---|
| | **e** Click the **Close Data File** icon in the main toolbar, and click **Close** when the dialog box containing the list of data files pops up. | • The product ions appear to be:<br>Sulfamethizole-271.0 > 155.7<br>Sulfamethazine-279.0 > 185.8<br>Sulfachloropyridazine-285.0 > 155.7<br>Sulfadimethoxine-311.0 > 155.7 |

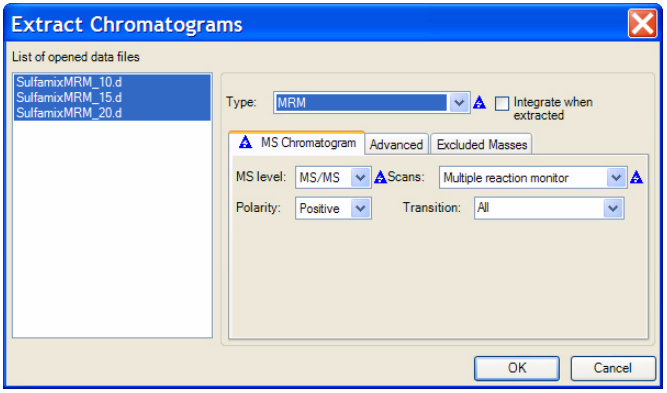
## Task 5. Find optimum collision energy for MRM acquisition

In this task, you set up MRM acquisition methods for the sulfa drugs for different collision energies. By examining the spectra and comparing peak intensities, you determine the optimal collision energy settings for the compounds.

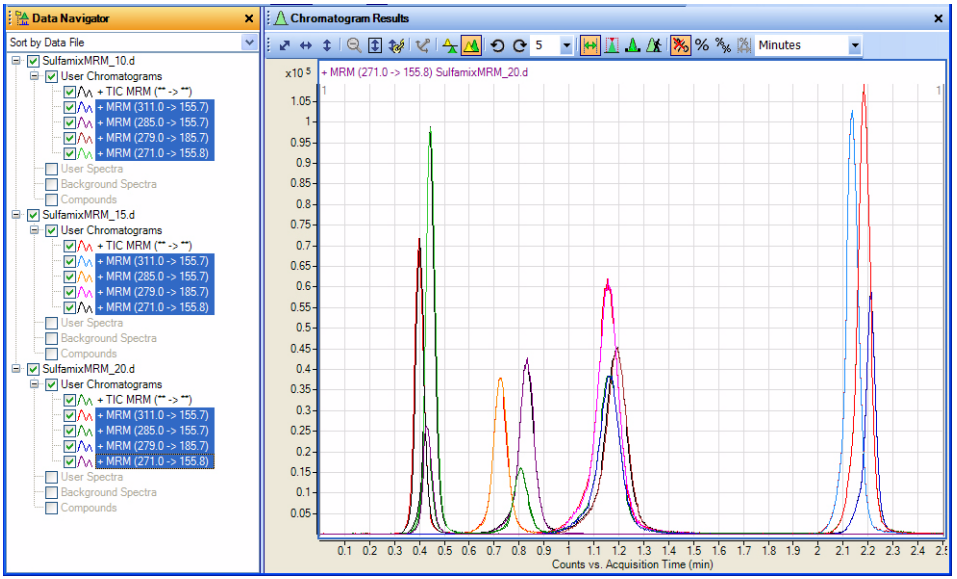
| Steps | Detailed Instructions | Comments |
|---|---|---|
| **1** Set up three MRM acquisition methods.<br>• Use all the MS parameters in the example below except for the collision energy value.<br>• Use collision energies of 10, 15 and 20.<br>• Save methods as ***iii*Sulfamix MRM_*xx*.m**, where ***iii*** are your initials and ***xx*** is the collision energy. | **a** Click the **MS QQQ** tab.<br>**b** Set **Scan Type** to **MRM**.<br>**c** Enter all MS parameters shown in the example below except for the collision energy value.<br>**d** In the collision energy column, type `10` for each compound.<br>**e** Save the method as ***iii*Sulfamix MRM_10.m**.<br>**f** Repeat step d and step e for collision energies of 15, 20, saving the methods as ***iii*Sulfamix MRM_*xx*.m**, where ***iii*** are your initials and ***xx*** is the collision energy. | • Because the largest peaks were produced with a collision energy of 15 in the previous exercise, you will look at only those collision energies to either side of 15. |




| | | |
|---|---|---|
| **2** Set up and run the worklist (optional).<br>• Specify the data files as ***iii*Sulfamix MRM_*xx*.d**, where ***iii*** are your initials and ***xx*** is the collision energy. | **a** Click the **Worklist** icon if necessary to make sure the worklist is visible.<br>**b** Add three samples to the worklist for collision energies 10, 15, 20.<br>**c** Mark the checkbox to the left of the Sample Name for each of the three samples.<br>**d** Click **Run > Worklist**. | • This step is optional because you can use the six example data files in the next step. |

| Steps | Detailed Instructions | Comments |
|---|---|---|
| **3** Compare the compound transition intensities at different collision energies.<br>• Open the MRM data files:<br>SulfamixMRM_10.d<br>SulfamixMRM_15.d<br>SulfamixMRM_20.d<br>• Set the MRM chromatogram extraction parameters as shown at right for all transitions.<br>• Disable the TICs for clarity and examine the peak intensities.<br>• Compare the intensities of each compound transition obtained at one collision energy with the same compound transition obtained at another collision energy. (Do this in Overlaid Mode with all the MRM chromatograms.)<br>• Close the data files but don't save results.<br>• Refer to Table 3 on page 33 for optimal method settings for each compound. | **a** Open the **Qualitative Analysis** program.<br>**b** Clear the **Run 'File Open' actions...** check box.<br>**c** Open the MRM data files in the Qualitative Analysis program.<br>**d** Right-click the Chromatogram Results window, and click **Extract Chromatograms** from the shortcut menu.<br>**e** To select all data files, click the last file while holding down the **Shift** key.<br>**f** Enter the parameters as listed in the example below, and click **OK**.<br>**g** Clear the TIC check boxes to make the MRM chromatograms easier to view.<br>  | • Why a spectrum for MRM? It's a feature of the program to show spectra even for MRM experiments and can be quite handy for comparing relative intensities of product ions generated from the same precursor. |
| | **h** Click the **Overlaid Mode** icon, .<br>**i** Compare peak intensities for each compound transition in each data file in the Chromatogram Results window. | • Compare the colors shown in Chromatogram Results with the color next to the MRM transition name in Navigator. |

| Steps | Detailed Instructions | Comments |
|---|---|---|




Unless you decide to acquire MRMs at lower collision energies, you should find that the optimal method settings are as shown in Table 3.

j Click the **Close Data File** icon in the main toolbar, and click **Close** when the Close Data File dialog box appears.

- You now have all the information you need to do an MRM acquisition experiment of the sulfa drug mixture. Consider doing at least one more run with those settings.

**Table 3**

| Compound Energy | MRM Transition | Fragmentor | Collision |
|---|---|---|---|
| Sulfamethizole | 271.0 > 155.8 | 100 V | 10 |
| Sulfamethazine | 279.0 > 185.7 | 100 | 15 |
| Sulfachloropyradizine | 285.0 > 155.7 | 100 | 10 |
| Sulfadimethoxine | 311.0 > 155.7 | 100 | 20 |

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## In This Book

This exercise helps you use the Agilent 6410 Triple Quad LC/MS system. In this guide you learn how to develop an acquisition method.

If you have comments about this guide, please send an e-mail to feedback_lcms@agilent.com.

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